In Vivo Thrombogenicity of Embolic Protection Systems for Angioplasty and Stenting

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Key words: thrombogenicity, embolic protection devices, angioplasty

Summary

Despite the increasing use of embolic protection systems (EPS) for carotid stenting, their intrinsic in vivo thrombogenicity remains unknown. We studied three different types of EPS (n = 24) deployed in the carotid arteries of pigs in which pools of platelets and fibrinogen were labelled with ^{111}In and ^{125}I. The amount of clot deposition seen on photography was also scored using a qualitative scale.

EPS made of fabric nets under normal flow conditions were 5-6 and 15-16 times more thrombogenic (for both platelet (P=.04) and fibrin (P=.007)) than Nitinol mesh nets. Clot deposition on Nitinol mesh nets was more abundant under flow arrest than under normal flow conditions (P=.018).

EPS differ in intrinsic thrombogenicity, a characteristic of the material that could be investigated in pre-clinical studies designed to optimize devices.

Introduction

The most important complication of carotid angioplasty and stenting is embolic occlusion of cerebral vessels. Thus protection devices have been specifically designed for this procedure. Despite their common use in clinical practice, there is no documentation of their intrinsic thrombogenicity, which may entail clinical risks in itself [1]. The goal of the present work was to compare the in vivo thrombogenicity of commercially available carotid protection devices.

Material and Methods

All experimental procedures were approved by our Institutional Animal Care and Use Committee and performed in compliance with guidelines of the Canadian Council on Animal Care. All procedures were performed under general anaesthesia.

^{111}Indium-oxine platelet and ^{125}I-fibrinogen pools labelling

Venous blood samples (35 ml) from each animal were collected in syringes containing 1/10 of 3.8% citrate buffer. Blood samples were gently mixed and processed without delay. Platelet rich plasma (PRP) was prepared by slow centrifugation (200 g for 10 min). An equal quantity of modified tyrode solution (MTS: tyrode, heparin and water, 28:1:1, pH 6.2-6.5) was added to the PRP, and the mixture was centrifuged at 1000 g for ten minutes.

The platelet pellet was then separated from platelet poor plasma (PPP), suspended in 8 ml of MTS and incubated at 37°C for two minutes with 500 mCi of ^{111}Inoxine (Amersham Health, Mississauga, Canada). Five ml of PPP were then added and the solution was centrifuged at 1000 g for ten minutes. The supernatant was re-

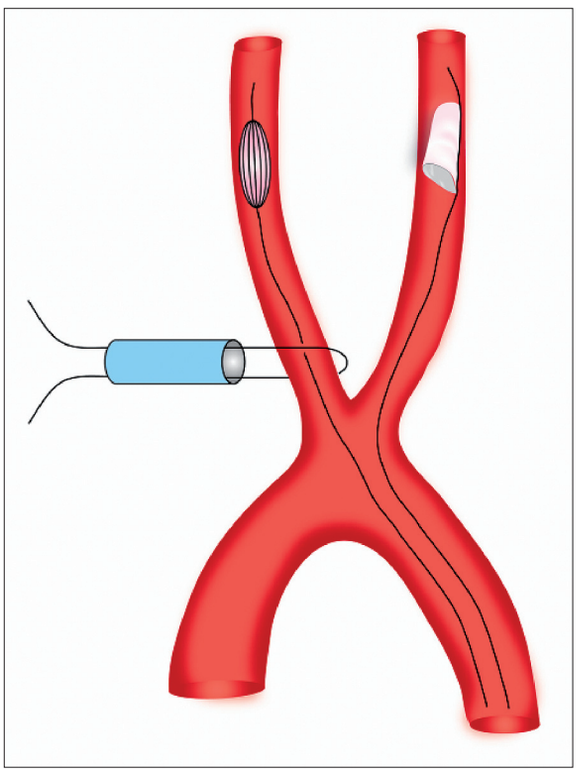

Figure 1 Illustration of animal model. Devices were compared in a paired, simultaneous fashion.

moved, and radioactivity was measured for the supernatant and for an aliquot of the labelled platelets, to determine labelling efficacy (LE (%) = Platelet radioactivity X 100 / Total radioactivity)[2]. Labelled platelets were re-injected immediately. The fibri-nogen pool was labelled by the i.v. injection of 2 mg (220µCi) of 125I-fibrinogen (Amersham Biosciences) in each animal.

Animal experiments

Four animals were used for this study. Animals were anticoagulated with 10000U of unfractionated heparin but no antiplatelet regiment was administered. Validation of the method was performed in two animals by comparing the thrombogenicity of three kinds of guide wires (0.035' stainless steel uncoated (Cook), coated(Terumo), coated but mechanically abraded with forceps). Two animals were used to evaluate protection systems (n = 24; 12 Nitinol mesh nets, six fabric nets, six fabric umbrellas, gifts from MicroTherapeutics, Boston Scientific and Cordis respectively). Bilateral arterial femoral sheaths and guiding catheters (8F) were inserted to introduce and retrieve devices from both carotid arteries in a repeated fashion, to perform a paired and simultaneous comparison of two devices, each side being dedicated to the study of one kind of device, each animal to a pair-wise comparison of two devices (figure 1). Six samples of each device were deployed for one minute each time. This time was shown sufficient yet capable of discrimination of devices, and short enough to prevent the accumulation of a sizable clot on thrombogenic wires in preliminary studies. In two animals Nitinol mesh nets were tested under normal flow conditions and under flow arrest by carotid occlusion using a tourniquet around the proximal common carotid artery, under angiographic control, to mimic conditions of occlusion during angioplasty, platelet and fibrin deposition presumably occurring differently according to blood flow. Animals were sacrificed by barbiturate overdose at the end of the experiment. Once retrieved, devices were severed from the pusher wire, samples were counted and immersed into formalin and photographed. Quantification of platelet and fibrin deposition was performed by counting each device in a multi-channel counter, correcting for the other isotope (5.57% of ^{125}I counts could be attributed to ^{111}In). In each animal two devices were compared using paired Student's t tests (results between animals cannot be compared because of varying degrees of platelet and fibrinogen pool labelling).

Scoring of stereographs

Devices were stereographically photographed and the amount of clot deposition scored using a qualitative scale. A score of I indicated a normal appearance, II minimal deposition, and III a major accumulation of material. Scores were compared by using a Mann-Whitney test. A P value less than .05 was considered a significant difference.

Results

The platelet-labelling efficacy of ^{111}In-oxine varied from 45.6 to 70% with a mean value of 58%. Relative platelet adhesion and fibrin deposition were nine times and 18 times more abundant on stainless steel uncoated guide wires compared to the coated wires respectively (P=.05 and P=.005 respectively; figure 2).

There was no relationship between thrombogenicity, as assessed by labelling methods or stereophotography, and order of deployment of devices inside carotid arteries.

Fabric nets, under normal flow conditions, were five to six (for platelet (P=.04) and 15-16 times (for fibrin (P=.007)) more thrombogenic than Nitinol mesh nets (figure 2).

There was no significant difference between Nitinol mesh nets and fabric umbrella nets. Material was more abundant on fabric nets than on Nitinol mesh nets (P=.009).

Qualitative scores of stereophotographs showed more abundant material on Nitinol mesh nets in flow arrest (median score III) as compared to normal flow conditions (Median score II; P=.018; figure3). Clots were also frequently noted to be outside as well as inside devices (figure 3).

Discussion

There is no consensus on in vitro models that can reliably predict the clinical thrombogenicity of materials and the coagulation cascade and platelet functions differ widely between species, without any definite indication regarding which is most likely to reproduce the human context [3,4].

The biological meaning of our experiment is supported by a number of facts. The platelet/ fibrin ratio observed on devices consistently differed from whole blood, suggesting that the experiment underscores a phenomenon different from passive deposition of blood. As expected in the arterial circulation, platelet adhesion was relatively more important (approximately 5-10 x) than fibrin deposition, as compared to whole blood ratios [5]. Standard objects led to differences in the expected direction; coated surfaces showing decreased thrombogenicity as compared to uncoated surfaces, and devices tested under flow arrest showing more deposition than under normal flow conditions. Finally the presence of material both inside and outside "protection devices" (figure 3) suggests concerns for potential device-related emboli during clinical use.

Weaknesses of the method include: the

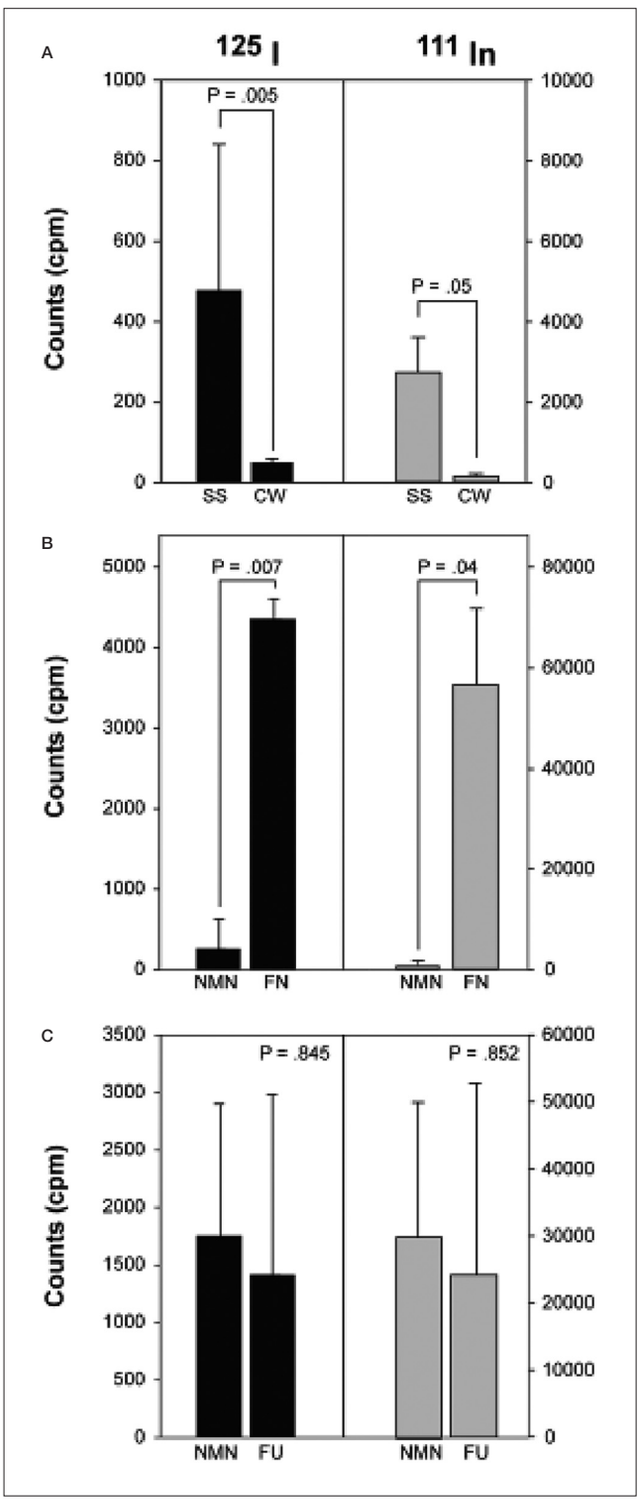

Figure 2 Intrinsic thrombogenicity. ^{111}In-platelet (right) and ^{125}I-fibrinogen (left column) counts of devices are illustrated in a comparative fashion. A) CWs were less thrombogenic than SSs, B) Nitinol mesh nets were less thrombogenic than FNs in animal 3. C) There was no significant difference between Nitinol mesh nets and FUs in animal 4.

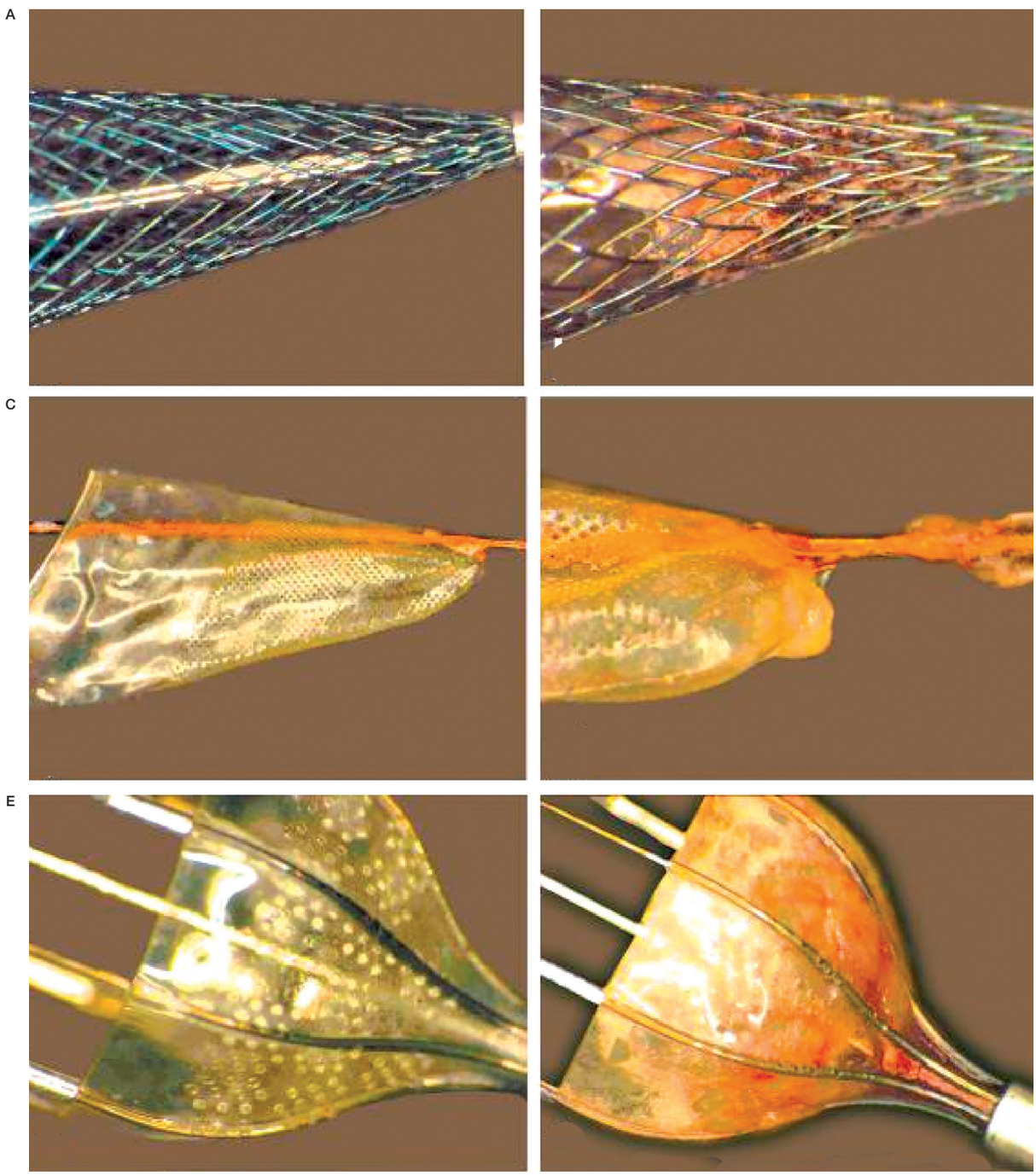

Figure 3 Stereophotographs of devices. Examples of devices showing better (left) or worse (right) scores on macroscopic examination. Nitinol mesh nets showed a higher median score when tested under carotid occlusion (B) than in normal flow condition (A). Fibrin or mixed clots were commonly found inside and outside protection devices (C-F).

small numbers of animals; wide variations; potential loss of material during capture inside the delivery systems; the possibility of variable "shielding" of radiation by materials that differed from one object to another. Additional work could also focus on the effects of various antiplatelet regimens, since these are usually included in clinical protocols. This type of work,

independent from the Industry, is however limited by the number of expensive devices that are available.

Activities recovered on devices should be related to the surface areas, but we did not compensate for this factor. The design of the device, in addition to the material itself, must have some impact on thrombogenicity. A priori one may expect that as "filtering" efficacy increases with surface area, so does intrinsic thrombogenicity.

The use of carotid protection devices has been empirical, but increasing in popularity. The current work suggests that preclinical work could be included in the design of protection devices, but a valid trial is still necessary to prove that these tools are associated with benefits to patients.

Conclusions

Protection devices have an intrinsic thrombogenicity that varies with materials and designs.

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Acknowledgment We wish to thank Lysanne Normandeau, CHUM Radiation safety officer, for monitoring the safe handling of radioactive material, and for her advice on analyses. This study was supported by grants from Canadian Institutes of Health Research (MOP-44062) and the Heart and Stroke Foundation of Canada.